A Human *In Vivo* Model for the Determination of Lead Bioavailability Using Stable Isotope Dilution

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Beverages stored in lead-crystal glass accumulate extraordinary concentrations of lead. We obtained a lead-crystal decanter manufactured with lead from Australia, where the ratio of $^{206}Pb/^{207}Pb$ is distinctly different from that in the United States. We sought to determine the bioavailability of crystal-derived lead, using the technique of stable isotope dilution in blood. We conducted a single-dose, nonrandomized cross-over study in which participants were admitted to the Clinical Research Center twice, 1 week apart. During the first admission, subjects ingested sherry obtained from the original bottle. During the second admission, they ingested sherry that had been stored in the crystal decanter and that had achieved a lead concentration of 14.2 µmol/l. After ingesting decanter-stored sherry, mean blood lead rose significantly ($p = 0.0003$) from 0.10 to 0.18 µmol/l, while mean $^{206}Pb/^{207}Pb$ fell from 1.202 to 1.137 ($p = 0.0001$). On average, 70% of the ingested dose of lead was absorbed. We conclude that lead derived from crystal glass is highly bioavailable; repeated ingestions could cause elevated blood lead concentration. The technique of stable isotope dilution lends itself to the study of the bioavailability of lead in other matrices, including soil. *Key words*: alcohol, bioavailability, lead, lead isotopes, mass spectrometry, stable isotope dilution. *Environ Health Perspect* 104:176–179 (1996)

The contamination of soils with lead has created major remedial, legal, and policy problems in the United States and throughout the world. Contributions from industrial sources, mining, exterior paints, and leaded gasoline have created numerous sites where normal hand-to-mouth activity in children could result in significant lead ingestion. Across the United States, the Environmental Protection Agency (EPA) has found that of the 1300 sites on its National Priorities List, at least 922 of these sites contain lead (*1*).

Computer models aimed at estimating actual exposure use assumptions concerning lead bioavailability; i.e., the fraction of the ingested lead dose that enters the blood. We set out to create a human *in vivo* model in which trace quantities of lead in soils or liquids might be ingested and precise, site-specific estimates of bioavailability could be derived. Such estimates would greatly improve the precision of risk assessments. We chose to develop this model by first studying the bioavailability of lead in a matrix somewhat more palatable than soil, namely, sherry wine.

The bioavailability of lead in wine is a parameter of interest. In 1991, we observed that the lead concentration of port wine in three different decanters rose from 0.43 µmol/l to 10.43, 14.77, and 25.73 µmol/l (*2,3*). Alcoholic beverages from decanters in the homes of our colleagues were found to contain lead concentrations ranging from 6.77 to 103.90 µmol/l. We wondered why the literature contains no case reports of lead toxicity attributable to lead-crystal glassware and considered the possibility that the bioavailability of lead in wine might be exceedingly low.

A host of biological and chemical factors influence the bioavailability of lead in humans (*4*). For example, among adult volunteers, 9.7% of dietary ^{204}Pb was absorbed (*5*). Other studies of radioactive ^{203}Pb in drinking water (without food) found absorption to range from 60 to 70% (*5*); absorption of ^{203}Pb in water was lessened when ingestion occurred in proximity to a meal (*6*). The fraction of lead absorbed from wine is unknown, but it has been reported (in animals) that alcohol facilitates lead absorption (*7–9*) while tannins inhibit it (*10*).

Lead has four stable isotopes, ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb, of which the last three are continually being produced by radioactive decay. Thus, lead in industrial use can vary greatly in its stable isotope ratios according to the geological age of the lead deposit. For example, the ratio of $^{206}Pb/^{207}Pb$ in the Broken Hill, Australia, lead deposit is 1.03; in the younger U.S. deposits of the Mississippi valley, it is as high as 1.42 (*11*). We had in our possession a crystal decanter that had a $^{206}Pb/^{207}Pb$ ratio of 1.078, clearly manufactured with lead of Australian origin. We realized that the bioavailability of lead in wine or spirits stored in this decanter could readily be determined by isotope dilution in American-born subjects whose $^{206}Pb/^{207}Pb$ ratios average approximately 1.200. We further realized that the bioavailability of lead in any matrix could be determined by isotope dilution provided the isotopic ratio of the ingested sample is sufficiently different from that in the blood of the subjects who ingest it. The advantage of this technique is its extraordinary sensitivity and precision, which allows one to estimate bioavailability even when the fraction of lead absorbed is as low as 5%. Thus, we also examined the blood $^{206}Pb/^{207}Pb$ ratios of subjects living in New York, Scotland, and Australia to develop a cadre of potential study volunteers for this and other studies.

Methods

In New York, this study was approved by the Columbia Presbyterian Medical Center (CPMC) Institutional Review Board, and written informed consent was obtained from each subject. In Glasgow, Scotland, and in Brisbane, Australia, the sampling of blood was approved by the Research and Ethical Committee of the Western Infirmary and the Ethical Committee of Princess Alexandra Hospital, respectively.

In New York, 61 adult volunteers donated 10 ml of blood in response to advertisements posted around the Health Sciences Campus of Columbia University. In Glasgow and Brisbane, blood was obtained from 10 and 12 subjects, respectively.

This was a single dose, nonrandomized, cross-over study. Of the 61 subjects screened for $^{206}Pb/^{207}Pb$ in New York, 6 healthy women and men were chosen who had blood lead concentrations (BPbs) <0.24 µmol/l and blood $^{206}Pb/^{207}Pb$ ratios ≥1.195. All were between the ages of 21 and 40 years and were

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within 10% of their ideal weight for height according to the Metropolitan Life Tables. The six subjects were screened before the study to assure that they had normal urinalyses, blood chemistries, blood counts, and physical exams. Subjects were admitted to the Irving Center for Clinical Research (day 1), received a regular hospital dinner, and were then fasted overnight, except for water. At 0600, 0630, and 0700 hr on day 2, baseline BPb and $^{206}Pb/^{207}Pb$ samples were taken; at 0700 hr blood for δ-aminolevulinic acid dehydratase (ALAD) was also taken.

Immediately following the 0700 hr blood drawing, subjects consumed approximately 100 ml of sherry. An initial control hospitalization involved the administration of a glass of sherry obtained directly from the original bottle, with a lead content of 0.28 µmol/l. During a second admission, subjects drank sherry that had been stored for 3 years in a lead crystal decanter and had reached a lead concentration of 14.24 µmol/l; a dose of 1.21 µmol Pb/70 kg body weight (250 µg Pb/70 kg) was administered. Blood samples for $^{206}Pb/^{207}Pb$ and BPb were then taken at intervals over the next 48 hrs. Urine samples were also collected at intervals before and after ingestion.

The subjects received no breakfast and had a standardized liquid lunch at noon. At 1700 hr on day 2, they received a standardized dinner, followed by a standardized breakfast, lunch, and dinner on day 3. All meals were prepared in duplicate and one portion was homogenized and frozen for analysis; meals were unremarkable for lead content (all <0.5 ppm Pb dry weight).

The ratio of $^{206}Pb/^{207}Pb$ and total lead content were simultaneously measured in the blood and the urine. An appropriate quantity of ^{205}Pb was added as an internal standard to each blood sample, which was then decomposed by boiling in concentrated HNO_3. The solution was evaporated to dryness and the residue charred at 250°C. Lead was then extracted by anion exchange chromatography and isotopically analyzed on a Finnigan MAT 261 multicollector mass spectrometer (*12*). For the admission involving the consumption of ordinary sherry, BPb was measured by atomic absorption spectrophotometry according to the method of Fernandez and Hilligoss (*13*). ALAD, a measure of ongoing metabolic lead toxicity, was assessed using a colorimetric enzyme assay (*14*). All other blood and urine analyses were performed by the CPMC laboratories.

Results

The frequency distributions of blood $^{206}Pb/^{207}Pb$ ratios for subjects screened in New York, Brisbane, and Glasgow are illustrated in Figure 1. Most New York subjects had blood $^{206}Pb/^{207}Pb$ ratios of 1.195 or higher; those who had lower ratios typically had spent a considerable portion of their lives outside the United States. Australian subjects had the lowest $^{206}Pb/^{207}Pb$ ratios, ranging from 1.095 to 1.145, while Scottish subjects had only slightly higher values, ranging from 1.105 to 1.155.

The demographic characteristics of the six New York subjects selected for the wine ingestion study are presented in Table 1.

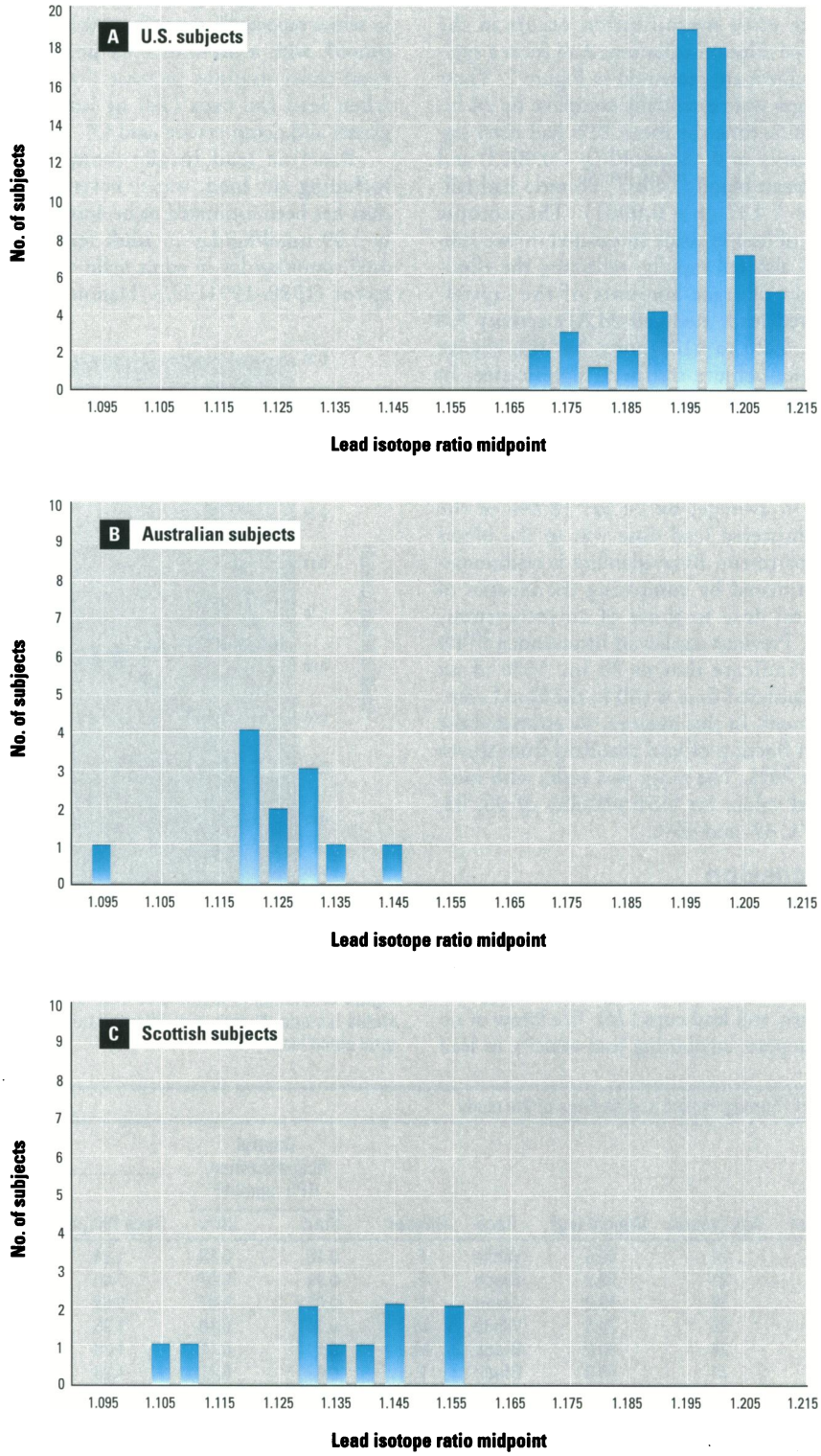

Figure 1. Frequency distribution of $^{206}Pb/^{207}Pb$ isotope ratios in normal volunteers from (A) Columbia University, New York, (B) Brisbane, Australia, and (C) Glasgow, Scotland.

Prior to the ingestion of sherry that had been in a lead-crystal decanter ($^{206}Pb/^{207}Pb$ = 1.078), the mean BPb was 0.10 µmol/l and the mean $^{206}Pb/^{207}Pb$ ratio was 1.202. After consumption of the sherry, the BPb rose and the $^{206}Pb/^{207}Pb$ ratio fell to attain values in the range 1.118–1.150 at 48 hr, bracketing the value (1.135) at which minimum error magnification occurs in the isotope dilution equation; data from a typical subject are presented in Figure 2. These changes were essentially complete by 24 hr, at which time the mean BPb had risen significantly to 0.18 µmol/l (p = 0.0003) and the mean blood $^{206}Pb/^{207}Pb$ ratio had fallen to 1.137 (p = 0.0001). The isotopic ratio of lead in urine (measured in two subjects) also fell rapidly, reflecting the elimination of trace amounts of the crystal-derived lead. Red cell ALAD activity fell from 4520 to 4183 units, but this change did not achieve statistical significance. In contrast, neither BPb (Table 1), blood $^{206}Pb/^{207}Pb$ ratio, nor ALAD changed significantly after the consumption of sherry obtained from the original bottle.

On average, by 24 hr, 38.4% of the administered lead dose was in the blood compartment. Bioavailability is traditionally estimated by comparing the kinetics of an oral dose to those of an intravenous dose. Previous studies of intravenous ^{203}Pb (*15*) indicate that at 24 hr, 55% of an administered dose is still in the blood compartment. In this manner, we estimated the mean fraction of lead absorbed from sherry to be 70%. The range was wide, with individual values for bioavailability of 96, 83, 77, 71, 47, and 46%.

Discussion

Historically, lead has found its way into wines due to its use as a sweetener and from products such as leaden kettles, distillation devices, basins for mulling wine, lead-glazed pottery, and lead cups (*16*). We know of no case reports attributing lead toxicity to lead crystal glassware, although cases involving contamination from other sources have been reported (*17*). Epidemiologic studies have found a dose–response relationship between alcohol consumption and BPb (*18–22*); wine consumption has been reported as a specific risk factor (*18,19*). This is not surprising as lead concentrations in wines reportedly range from 0.14 to 1.21 µmol/l, with a mean of 0.42 µmol/l (*23*). Lead concentrations increase dramatically when lead foil caps (*24*) or lead-crystal glasses and decanters are used (*3*).

Baseline lead intake from sources including air, food, water, beverages, and dust has been estimated to be approximately 3.09 nmol/kg/day in adult females and 3.67 nmol/kg/day in adult males (*25*). The recent (1988–1991) U.S. National Health and Nutrition Examination Survey reported mean BPbs of 0.12 and 0.19 µmol/l for those aged 20–49 and 50–69 years, respectively (*26*). The contribution of lead from any particular source to BPb is obviously related to the dose ingested, the fraction absorbed, and the regularity of ingestion. Estimates of approximately 0.10 µmol/l BPb for each 0.48 µmol/day of dietary lead intake, and 0.29 µmol/l BPb for each 0.48 µmol/l of lead in the water supply have been reported (*27*); these estimates assume repeated daily ingestion. Several investigators have concluded that among regular wine drinkers, lead intake from wine may exceed that from diet, water, air, and dust combined (*23,24,27,28*). We concur, and conclude that for a 70-kg subject, a single dose of 0.48 µmol of lead in sherry will

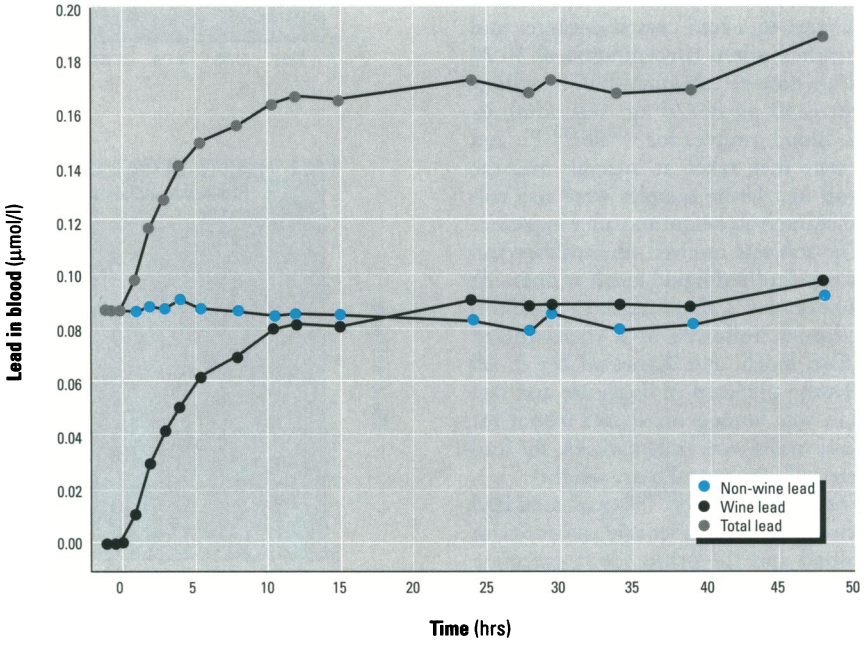

Figure 2. Change in blood lead after ingestion of sherry containing 1.21 µmol/Pb/70 kg body weight. By stable isotope dilution, we differentiated the relative contributions of wine lead and endogenous lead to total blood lead.

Table 1. Demographics and findings of the study

					Control hospitalization, BPb (µmol/l)[a]		Lead crystal hospitalization					
								BPb (µmol/l)[b]		$^{206}Pb/^{207}Pb$		
Subject	Age (years)	Weight (kg)	Race	Gender	Start	24 hr	Dose Pb (µmol)	Start	24 hr	Start	24 hr	% Absorbed
1	24	60.6	White	F	0.16	0.16	1.04	0.17	0.29	1.206	1.148	96
2	23	58.2	Black	F	0.09	0.07	1.00	0.06	0.16	1.202	1.120	83
3	28	48.0	Asian	F	0.06	0.07	0.83	0.07	0.14	1.204	1.144	46
4	26	73.5	White	M	0.10	0.10	1.26	0.10	0.19	1.199	1.135	77
5	24	80.0	Black	M	0.11	0.11	1.38	0.08	0.14	1.196	1.142	47
6	24	60.0	Black	F	0.09	0.11	1.03	0.09	0.17	1.202	1.135	71
Mean	25	63.4			0.10	0.10	1.09	0.10	0.18*	1.202	1.137**	70

[a]Analyzed by atomic absorption spectrophotometry.
[b]Analyzed by isotope dilution mass spectrometry.
*p = 0.0003, compared to starting BPb.
**p = 0.0001, compared to starting $^{206}Pb/^{203}Pb$ ratio.

induce a rise in BPb of approximately 0.05 µmol/l. The probability that one might ingest a sufficient dose to induce clinically recognizable plumbism is small, but not zero, as beverages with more than 96.52 µmol/l have been found in homes (*2*).

Strictly speaking, our study has examined the bioavailability of the lead attributable to lead crystal. It is possible that the bioavailability of wine lead derived from other sources might be slightly different. Also, due to cost constraints, we could not evaluate the impact of food on the bioavailability of lead in wine.

Our subjects were carefully screened to have initial BPbs of <0.24 µmol/l and were administered a dose of lead that would not cause the BPb to rise above 0.48 µmol/l, even if absorption were complete and instantaneous. Through this approach, the stable isotope dilution method can also readily be applied to the study of lead in other matrices.

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